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Indian Standard

LIQUID SUGAR — SPECIFICATION

ICS 67.180.20

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

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Sugar Industry Sectional Committee, FAD 02

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Sugar Industry Sectional Committee had been approved by the Food and Agriculture Division Council.

Liquid sugar is prepared by the hydrolysis of sucrose to glucose (dextrose) and fructose. This is achieved by subjecting a sucrose solution to enzymatic reaction. Liquid sugar syrups can be fully inverted as above, or partially inverted to leave part of the original sugar unchanged. These syrups are known as medium or partial liquid sugar syrups. Liquid sugar is an equimolar mixture of glucose and fructose obtained on hydrolysis of sucrose, because of its functionally more desirable properties like high solubility, high osmotic pressure, humectants nature and higher sweetness index as compared to cane sugar (sucrose), liquid sugar is a preferred substitute for cane sugar in most applications. This standard would help in defining the quality of liquid sugar in a manner that would enable better quality control. Liquid sugar is used for confectionery, beverages, bakery, biscuits, food and pharmaceutical formulations.

In the formulation of this standard due consideration has been given to the provision of *Legal Metrology (Packaged Commodities) Rules*, 2011 and *Food Safety* and *Standards Act*, 2006 and regulations framed thereunder. However, this standard is subject to restrictions imposed under this act, wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value observed or calculated, expressing the result of a test or analysis shall be rounded off in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

LIQUID SUGAR — SPECIFICATION

1 SCOPE

This standard prescribes the requirements and the methods of sampling and analysis for liquid sugar.

2 REFERENCES

The following standards contains provisions, which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standards indicated below:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
4706 (Part 2) : 1978	Method of test for edible starch and starch products: Part 2 Chemical method
14350 : 1996	Code for hygienic conditions in sugar factories
15279 : 2003	Sugar and sugar products, method of tests

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Raw Sugar — Unwashed centrifugal sugar with a minimum polarization of 96.5 °Z; surrounded by the original film of molasses; derived from sugarcane or sugar beet; to be further refined or reprocessed for making it suitable for direct consumption sugar /liquid sugars.

3.2 Liquid Sugar — Liquid sugar is virtually colourless /pale straw /golden coloured liquid sucrose syrup produced from raw sugar/ plantation white sugar/ cane sugar, etc, to meet the standards defined in this standard.

4 GRADES

Liquid sugar shall be of of two grades. The grades shall be classified as Grade 1 and Grade 2.

5 REQUIREMENTS

5.1 The liquid sugar shall be in the form of odourless and viscous syrup with a characteristic sweet taste. It shall be clear, free from fermentation, mould growth, sediment, dirt or other suspended and extraneous matter, or added sweetening and flavouring agents.

5.2 The product shall also comply with the requirements given in Table 1 and Table 2.

6 HYGIENIC CONDITIONS

The product shall be manufactured, handled, stored and packed under hygienic conditions (*see* IS 14350). The basic principle of hygiene underlining the technical advice in this standard should be applied with appropriate modification.

7 PACKING AND MARKING

7.1 Packing

The material shall be packed in clean, dry and leak proof containers. Unless otherwise agreed to between the purchaser and the vendor, such containers may either be lacquered lined steel drums or tin containers.

Table 1 Requirements for Liquid Sugar

(*Clause 5.2*)

Sl No.	Characteristics	Requirements		Method of Test, Ref to	
		Grade 1	Grade 2	Relevant Clause of Other Indian Standards	Annex
(1)	(2)	(3)	(4)	(5)	(6)
i)	Total solid percent (Brix percent), *Min*	66.00	66.00	—	A
ii)	Polarization, *Min*	99.0°Z	97.5°Z	**5** of IS 15279	—
iii)	Invert sugar percent by mass, *Max*	0.50	1.00	—	B
iv)	Conductivity ash, percent by mass, *Max*	0.06	0.10	**9** of IS 15279	—
v)	*p*H	6.5 - 7.2	6.5 - 7.2	**13** of IS 4706 (Part 2)	—
vi)	Colour in ICUMSA units, *Max*	200	1 000	**8** of IS 15279	—
vii)	Sulphur dioxide, mg/kg, *Max*	10	10	**13** of IS 15279	—

Table 2 Microbiological Requirements for Liquid Sugar

(*Clause* 5.2)

Sl No.	Characteristics	Requirements		Method of Test, Ref to Annex
		Grade 1	Grade 2	
(1)	(2)	(3)	(4)	(5)
i)	Yeast per10 g (dry solids), *Max*	10	10	C
ii)	Moulds per10 g (dry solids), *Max*	10	10	C
iii)	Mesophilic bacterial count per 10 g (dry solids), *Max*	200	200	D

7.2 Marking

The containers shall be suitably marked with the following information:

a) Name of the product;
b) Grade of the material;
c) Name and address of the manufacturer;
d) Batch or Code number;
e) Date of manufacture;
f) Net quantity of the content;
g) The words 'Best before .... (month and year to be indicated);
h) Direction for storage; and
j) Any other information required under the *Legal Metrology (Packaged Commodities) Rules*, 2011 *and Food Safety & Standards (Packaging and Labelling) Regulation*, 2011.

7.3 BIS Certification Marking

The product may also be marked with the Standard Mark.

7.3.1 The use of the Standard Mark is governed by the provisions of *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations framed thereunder. The details of the conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

8 SAMPLING

The method of drawing representative samples of the material and the criteria for conformity shall be as given in Annex E.

9 TESTS

9.1 The tests shall be carried out as prescribed in col 5 and col 6 of Table 1 and col 5 of Table 2.

9.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

ANNEX A

[*Table* 1, *Sl No.* (i)]

DETERMINATION OF TOTAL SOLIDS

A-1 EQUIPMENT AND APPARATUS

The following equipment and apparatus are required.

A-1.1 Diatomaceous Earth — Neutral to litmus when moistened with water. If a commercial grade is used, wash it by percolation with water which has been slightly acidulated with hydrochloric acid, until the effluent is acidic to litmus. Then rewash with water (not acidulated), until the effluent is neutral to litmus. Finally dry the earth in an oven at about 105°C.

A-1.2 Glass Stirring Rod, approximately 60 mm long with a flattened end.

A-1.3 Moisture Dish, with a cover made of aluminium; approximately 25 mm in height and 75 mm in diameter.

A-1.4 Desiccators, containing phosphorus pentoxide as desiccant.

A-1.5 Nickel Scoop

A-1.6 Vacuum Oven

A-2 PROCEDURE

Place about 10 g of the diatomaceous earth and the glass stirring rod in the moisture dish and dry the diatomaceous earth in an air oven at about 105°C. Cool in the dessicator and weigh. Repeat drying in the air oven, cooling in the dessicator and weighing till the mass is constant. Note the combined mass of the dish with the cover, the glass stirring rod and the dry diatomaceous earth. Weigh accurately about 5 g of the material in the nickel scoop; mix it with 5 ml of water and run on to the diatomaceous earth, using the glass stirring rod. Wash the scoop thrice, using 2 ml of water for each washing and transfer the washings on to diatomaceous earth, simultaneously working the contents of the moisture dish into a thick paste using the glass stirring rod. Leave the glass stirring rod in the dish. Place the moisture dish in the vacuum oven and dry the contents of dish at 100°C. Cool the dish and its contents in the dessicator and weigh. Repeat drying in the vacuum oven, cooling in the dessicator and weighing till the mass is constant.

A-3 CALCULATION

Total solids content,

$$\text{Percent by mass} = 100 - \left(\frac{100\,(M + A - B)}{M} \right)$$

where

M = mass of the material taken for the testing, in g;

A = mass of the moisture dish containing the glass stirring rod and dried diatomaceous earthing, in g; and

B = mass of the moisture dish containing the glass stirring rod, diatomaceous earth and the dehydrated material, in g.

ANNEX B

[*Table* 1, *Sl No.* (iii)]

DETERMINATION OF INVERT SUGARS

B-1 APPARATUS

B-1.1 Timer (1 h), to indicate minutes and seconds.

B-1.2 Fast Boiling Water Bath

B-1.3 Fine Pumice, or glass beads or anti-bumping granules.

B-1.4 Lead Ring, or other suitable weighting device.

B-1.5 Volumetric Flasks, capacities 100 ml, 250 ml.

B-1.6 Pipettes, capacities 25 ml, 50 ml.

B-1.7 Erlenmeyer Flask, capacity 300 ml.

B-1.8 Reflux Condenser

B-1.9 Burette, capacity 50 ml.

B-1.10 Beaker, capacity 300ml.

B-1.11 Filter Funnel

B-1.12 Analytical Balance, readable to mg.

B-2 REAGENTS

B-2.1 Carrez Solution I, dissolve in water 21.9 g of zinc acetate and 3 g of glacial acetic acid. Make up to 100 ml with water.

B-2.2 Carrez Solution II, dissolve in water 10.6 g of potassium ferrocyanide. Make up to 100 ml with water.

B-2.3 Methyl Orange, approximately 0.1 g/100 ml

B-2.4 Hydrochloric Acid, approximately 4.0 mol/l and approx. 1.0 mol/l.

B-2.5 Hydrochloric Acid, approximately 0.1 mol/l

B-2.6 Sodium Hydroxide Solutions, approximately 0.1 mol/l and 10 g/100 ml.

B-2.7 Luff Schoorl Reagent — Weigh about 86.2 g of sodium carbonate (anhydrous), 31.3 g of sodium bicarbonate, 70.0 g of sodium citrate and 25.0 g of cupric sulphate (powdered). Mix these dry salts well and dissolve the mixture while stirring constantly in 800 ml of cold water. Make up to 1 litre.

B-2.8 Sodium Thiosulphate Solution, 0.1 mol/l.

B-2.9 Starch Solution — Add a mixture of 5 g of soluble starch in 30 ml of water to 1 litre of boiling water. Boil for 3 min, leave to cool and if necessary add 10 mg of mercuric iodide as a preservative.

B-2.10 Sulphuric Acid, approxmately 3 mol/l.

B-2.11 Potassium Iodide Solution, 30 g/100 ml.

B-2.12 Granulated Pumice Stone, boiled in hydrochloric acid, washed in water and dried.

B-2.13 3-Methylbutan-1-ol, or liquid paraffin BP or silicone antifoam solution.

B-2.14 Phenolphthalein Solution, 1g/100 ml, prepare by dissolving 1 g of phenolphthalein in 60 ml of methylated spirit (denatured alcohol) and make up to 100 ml with water.

B-3 PROCEDURE

B-3.1 Standardization of the Luff Schoorl Reagent

Dissolve 2.375 g of sucrose (dried at 100°C) in about 100 ml of water in a 300 ml beaker, add 15 ml of 1 mol/l hydrochloric acid and sufficient water to give a volume of 150 ml. Heat to boiling point, boil for 2 min, cool, add 2 or 3 drops of phenolphthalein indicator solution, just neutralize with the 10 g/100 ml sodium hydroxide solution, transfer to a 500 ml graduated flask and dilute to 500 ml with water. This solution contains 0.005 g/ml of glucose equivalent.

Pipette 50 ml of this solution into a 200 ml graduated flask and dilute to the mark with water to produce a solution containing 1.25 mg/ml of glucose equivalent. Use 25 ml of this solution to carry out a Luff Schoorl titration according to the procedure given under **B-3.4**, including the blank titre. 31.25 mg of reducing sugar used shall produce a difference titre of 12.35 ml of 0.1 mol/l sodium thiosulphate. If the result is different from this, a standardization factor, *'F'* based upon glucose recovery should be calculated and all subsequent concentrations corrected accordingly as given under **B-4.1**.

B-3.2 Preparation of Clarified Molasses Solution

Weigh 2.5 g of molasses in a 250 ml flask and add 200 ml of distilled water. Clarify by adding 5 ml of Carrez solution 1 and stir for 1 min. Add 5 ml of Carrez solution II and again stir for 1 min. Bring to volume with cold water, mix and filter.

B-3.3 Preparation of Hydrolyzed Molasses

Using a pipette take 50 ml of the solution and transfer to a 100 ml volumetric flask. Add a few drops of methyl orange solution (*see* **B-2.3**) and stirring continuously, add hydrochloric acid (4mol/l) until the liquid turns a definite red. Add 15 ml of hydrochloric acid (0.1 mol/l), immerse the flask in a fast boiling water bath and keep there for 30 min. Cool rapidly to approximately 20°C and add 15 ml of sodium hydroxide solution (0.1 mol/l). Make up to 100 ml with water and homogenize. Remove not more than 25 ml containing less than 60 mg of reducing sugars expressed as glucose. If necessary make up to 25 ml with distilled water and then determine the content of reducing sugars by the Luff Schoorl method.

B-3.4 Titration by the Luff Schoorl Method

Using a pipette take 25 ml of Luff Schoorl reagent prepared as per **B-2.7** and transfer to a 300 ml of the hydrolyzed molasses solution (*see* **B-3.3)** and add several granules of pumice stone or anti-bumping granules. Fit a reflux condenser to the flask and place on a wire gauze under which a flame has been lit. Adjust the flame so as to bring the contents of the flask to the boil approximately 2 min. Rotate the flask occasionally by hand to keep the solution mixed. Boil for exactly 10 min, any tendency to foam may be controlled by a few drops of the appropriate antifoam reagent. Cool immediately in cold water and after 5 min titrate as follows: add 10 ml of potassium iodide solution and immediately afterwards (carefully because of the risk of abundant foaming), add 25 ml of 3 mol/l sulphuric acid. Titrate with 0.1 mol/l sodium thiosulphate solution until a dull yellow colour appears, add the starch indicator and complete the titration. Carry out the same titration on an accurately measured mixture of 25 ml of Luff Schoorl reagent (*see* **B-2.7**) and 25 ml of water, after adding 10 ml of potassium iodide solution and 25 ml of 3 mol/l sulphuric acid without boiling. This gives the blank titre with the difference titre being the titre of the hydrolysed molasses solution above less the blank titre.

NOTE — The reproducibility of the method depends upon the following precise procedures. For example, as per the procedure described under **B-3.3** it is essential that the flask is immersed in fast boiling water. The immersion of subsequent flasks must not be allowed to alter this and in case of procedure described under **B-3.4** the boiling time shall be precisely 10 min followed by rapid cooling.

B-4 CALCULATION

B-4.1 Calculation of Standardization Factor

Calculate the difference titre between the standardization titration and blank titration (*see* **B-3.1)** expressed, in ml, of 0.1 mol/l sodium thiosulphate solution. From the Table 3 determine the amount of glucose (in mg) equivalent to the difference titre interpolating, wherever necessary.

Let the amount of glucose determined from the Table 3 = x

Then the standardization factor, $F = \frac{31.25}{x}$

B-4.2 Calculation of Total Reducing Sugars

Calculate the difference titre between the sample titration and the blank titration (*see* **B-3.4**) expressed in ml of 0.1 mol/l sodium thiosulphate solution. From the Table 3 determine the amount of glucose (in mg) equivalent to the difference titre.

Let the amount of glucose determined from the Table 3 = *y*. Let the mass of sample taken in **B-3.2** = *m*

Then;

$$\text{Total reducing sugars} = \frac{2.0\,Fy}{m} \text{ percent } (m/m)$$

B-4.3 Precision

For molasses containing of the order of 50 percent total reducing sugars (range 40-60 percent), the absolute difference between two results obtained under repeatability conditions should not be greater than 1.64 percent.

The absolute difference between two results obtained under reproducibility conditions should not be greater than 2.59 percent.

Table 3 Table of Values for 25 ml of Luff Schoorl Reagent

(*Clause* B-4.1)

Sl No. (1)	Sodium Thiosulphate 0.1mol/L ml (2)	Glucose mg (3)
i)	1	2.4
ii)	2	4.8
iii)	3	7.2
vi)	4	9.7
v)	5	12.2
vi)	6	14.7
vii)	7	17.2
viii)	8	19.8
ix)	9	22.4
x)	10	25.0
xi)	11	27.6
xii)	12	30.3
xiii)	13	33.0
xiv)	14	35.8
xv)	15	38.5
xvi)	16	41.3
xvii)	17	44.2
xviii)	18	47.1
xix)	19	50.0
xx)	20	53.0
xxi)	21	56.0
xxii)	22	59.1
xxiii)	23	62.2

ANNEX C

[*Table* 2, *Sl No.* (i) *and* (ii)]

DETERMINATION OF YEASTS AND MOULDS

C-1 EQUIPMENT AND APPARATUS

C-1.1 Apparatus for Dry Sterilization at 180°C, for glassware sterilization.

C-1.2 Autoclave, **121 ± 1ºC**, for water and culture media sterilization.

C-1.3 Petri Dishes, of diameter 90-100 mm, sterile disposable plastic or glass, alternatively nutrient pad sets.

C-1.4 Pipettes, total delivery 1 ml; 10 ml graduated for dilutions.

C-1.5 Water Bath or Similar Apparatus, operating at 47 ± 1°C

C-1.6 Colony Counting Equipment.

C-1.7 pH Meter, accurate to ± 0.1 *p*H units at 25°C.

C-1.8 Test Tubes, for preparation of dilutions.

C-1.9 Erlenmeyer Flasks, 200 ml with a 100 ml mark.

C-1.10 Bunsen Burner

C-1.11 Incubator, operating at 30 ± 1ºC.

C-1.12 Bottle, for the medium.

C-1.13 Filter Funnel, base with support and clamp. These can be stainless steel, autoclavable plastic, glass or sterile disposable plastic, 50 m diameter with a capacity of 100 ml.

C-1.14 Flask for Mounting Filter Apparatus, 2 litre with side arm.

C-1.15 Vacuum Pump

C-1.16 Pipettes, 5 ml for use with nutrient pad method.

C-1.17 Forceps, for handling membranes.

C-1.18 Membranes, sterile 0.6 m pore size, 45 mm diameter.

C-2 DILUENTS, CULTURE MEDIA, REAGENTS AND OTHER PRODUCTS

C-2.1 Sterile Distilled Water, autoclaved at 121°C for 15 min.

C-2.2 Culture Media — Use one of the three following media:

a) *Wort agar (1litre)*:

Malt extract	15 g
Peptone	0.78 g
Maltose	12.75 g
Dextrin	2.75 g
Glycerol	2.35 g
Potassium hydrogen sulphate	1 g
Ammonium chloride	1 g
Agar	15 g
*p*H	4.8

b) *Yeast extract-glucose-chloramphenicol agar (1 litre)*:

Yeast extract	5 g
Glucose	20 g
Chloramphenicol	0.1 g
Agar	15 g
*p*H	6.6

NOTE — Oxytetracyline may be used instead of chloramphenicol, because oxytertracycline is heat-sensitive, it should be added to the medium just prior to use in the form of a filter-sterilized solution.

c) *Mycophil agar (1 litre)*:

Phytone peptone	10 g
Glucose	10 g
Agar	16 g
*p*H	4.0

Adjust the *p*H to 4.0 by adding 15 ml of sterile 10 percent (*v/v*) lactic acid to each litre of sterile melted medium prior to plating.

C-2.3 Disinfectant, for the working area.

C-2.4 Alcohol, for flaming the filter funnel assembly (*see* **C-1.13**).

C-3 SAMPLES

Collect representative samples in sterile containers, 200 ml for liquid sugar. Retain the samples until the analysis has been completed.

C-4 PROCEDURE

C-4.1 Preparation

Clean and disinfect the working area before commencing analysis. Mark the sterile Petri dishes with the date, the sample and the type of medium.

C-4.2 Medium Preparation

C-4.2.1 *Agar Medium*

Following the manufacturer's instructions carefully, rehydrate the culture medium with distilled or de-ionised water. Dispense the medium into bottles and sterilize at 121°C for 15 min.

Medium for immediate use should be cooled to 47°C in a water bath and dispensed into Petri dishes. Alternatively it should be allowed to solidify and be stored in the dark at 0-5°C for up to 1 month. Media can be re-melted.

C-4.2.2 *Nutrient Pads*

Wet the nutrient pads with 3-3.5 ml of sterile distilled or de-ionised water. Ideally a slight access of liquid should be visible at the edge of the pad.

C-4.3 Glassware Preparation

Sterilize the Erlenmeyer flasks with the plugs and the pipettes in the apparatus for dry sterilization (180°C) for 2 h.

C-4.4 Sample Preparation

Aseptically place 10 g of crystalline sugar or in the case of liquid sugar the amount corresponding to 10 g of dry matter, in a 200 ml flask. Erlenmeyer flask which is marked to indicate 100 ml. Add sterile water to the 100 ml mark, shake thoroughly to dissolve or mix the sugar solution.

NOTE — 20 g/100 ml must be taken for Canners sugar.

C-4.5 Inoculation and Incubation

Take two sterile Petri dishes. Transfer to each dish 1 ml of the initial sample solution (*see* **C-4.4**). If necessary prepare a dilution from the initial solution (10^{-1}). Adding 1 ml to 9 ml of sterile water in a test tube to give a series 10^{-2}, 10^{-3} and plate out similarly. Pour about 15 ml of the medium, previously melted and maintained at 47 ± 1°C in a water bath, from a culture bottle into each Petri dish. Carefully mix the inoculum with the medium and allow the mixture to solidify. Prepare a control plate with only 15 ml of the medium to check its sterility. Invert the plates and place them in the incubator at 30°C for 72 h.

C-4.6 Filteration and Incubation

When using the membrane filter method connect the filter apparatus to the vacuum pump. If not already sterile, flame the filter funnel and base with alcohol. After cooling place the membrane centrally on the base with sterile forceps. Place the sterile funnel on the top and clamp in place.

Pour the sample into the funnel, apply the vacuum and filter the sample. Rinse the funnel with sterile water. Turn off the vacuum. Remove the funnel and use flamed forceps to place the membrane on an agar plate or moistened nutrient pad. When positioning the membrane on the culture plate ensure that no air bubbles are trapped between them.

Invert the dishes and incubate at 30°C for 72 h.

C-5 EXPRESSION OF RESULTS

Count all the colonies from plates containing fewer than 150 colonies using the colony counting equipment. Yeasts are opaque, white, yellow or pink, whilst moulds form a mycelium which is often white with black, brown or green spores.

With colony counts below 10, the pour plate method is not practical. In this case the membrane filtration method is recommended.

Calculate the number of CFU per 10 g sugar or per 10 g of dry matter by the following calculation.

C-5.1 Plate Count

$$\frac{\Sigma c}{(n_1 + 0.1\, n_2)\, d} = \text{CFU/ g sugar or dry matter}$$

where

Σc = sum of the colonies counted on all the plates;

n_1 = number of plates counted in the first dilution;

n_2 = number of plates counted in the second dilution; and

d = dilution from which the first counts were obtained.

Example:

Two petri dishes of one dilution were incubated.

Dilution 10^{-2} Ist Plate = 99 CFU
2nd Plate= 114 CFU

$$\frac{99+114}{\left[2+(0.1\times 0)\right]\times 10^{-2}} = \frac{213}{0.02} = 10{,}650 \text{ CFU/g}$$

For 10 g of sugar or dry matter, the result is therefore 1.065×10^4 CFU/10g.

This is rounded to 1.1×10^4 CFU/10 g sugar or dry matter.

C-5.2 Membrane Filter

$$\frac{\Sigma c}{n} = \text{CFU/10 g of sugar or dry matter}$$

where

Σc = sum of the colonies on all the plates; and

n = number of plates.

ANNEX D

[*Table* 2, *Sl No.* (iii)]

DETERMINATION OF TOTAL MESOPHILIC BACTERIAL COUNT

D-1 EQUIPMENT AND APPARATUS

The following apparatus and glasswares are required.

D-1.1 Apparatus for Dry Sterilization at 170-180°C, for glassware sterilization.

D-1.2 Autoclave, 121 ±1°C, for water and culture media sterilization.

D-1.3 Incubator, operating at 30 ± 1ºC.

D-1.4 Bottle, for culture medium.

D-1.5 Filter Funnel, base with support and clamp. These can be stainless steel, autoclavable plastic, glass or sterile disposable plastic, 50 mm diameter with a capacity of 100 ml.

D-1.6 Flask for Mounting Filter Apparatus, 2 litre with side arm.

D-1.7 Vacuum Pump

D-1.8 Pipettes, 5 ml for use with nutrient pad method.

D-1.9 Forceps, for handling membranes.

D-1.10 Petri Dishes, 90-100 mm diameter, sterile disposable plastic or glass, alternatively nutrient pad sets.

D-1.11 Water Bath or Similar Apparatus, operating at 47 ± 1°C.

D-1.12 Colony Counting Equipment

D-1.13 Erlenmeyer Flasks, 200 ml with a 100 ml mark.

D-1.14 *pH* Meter, accurate to ± 0.1 *p*H units at 25°C.

D-1.15 Bunsen Burner

D-1.16 Membranes, sterile 0.45 µm pore size, 45 mm diameter.

D-2 DILUENTS, CULTURE MEDIA, REAGENTS AND OTHER PRODUCTS

D-2.1 Sterile Distilled Water

Autoclaved at 121°C for 15 min.

D-2.2 Culture Media

Use either nutrient agar or plate count medium as given below:

a) *Nutrient agar (1litre)*:

Beef extract	1 g
Sodium chloride	5 g
Yeast extract	2 g
Agar	15 g
Peptone	5 g
*p*H	7.5

b) *Plate count medium (1 litre)*:

Peptone	5 g
Yeast extract	2.5 g
Glucose	1 g
Agar	10 g
*p*H	7.0

Alternatively use:

Nutrient pad sets in petri dishes with membrane filters of 0.45 um pore size.

D-2.3 Disinfectant, for the working area.

D-2.4 Alcohol, for flaming the filter funnel assembly (*see* **D-1.5**).

D-3 SAMPLES

Collect representative samples in sterile containers, 200 ml for liquid sugar. Retain the samples until the analysis has been completed.

D-4 PROCEDURE

D-4.1 Preparation

Clean and disinfect the working area before commencing analysis.

Mark the sterile petri dishes with the date, the sample and the type of medium.

D-4.2 Medium Preparation

D-4.2.1 *Agar Medium*

Following the manufacturer’s instructions carefully, rehydrate the culture medium with distilled or de-ionised water. Dispense the medium into bottles and sterilize at 121°C for 15 min.

Medium for immediate use should be cooled to 47°C in a water bath and dispensed into Petri dishes. Alternatively it should be allowed to solidify and be stored in the dark at 0 - 5°C for up to 1 month. Media can be remelted.

D-4.2.2 *Nutrient Pads*

Wet the nutrient pads with 3-3.5 ml of sterile distilled or de-ionised water. Ideally a slight access of liquid should be visible at the edge of the pad.

D-4.3 Glassware Preparation

Sterilize the Erlenmeyer flasks with the plugs and the pipettes in the apparatus for dry sterilization (170-180°C). When the correct temperature is reached, sterilize for at least 1 h.

D-4.4 Sample Preparation

Aseptically place 10 g of crystalline sugar or in the case of liquid sugar, 10 g of dry matter, in a 200 ml flask. Add sterile water to the 100 ml mark, shake thoroughly to dissolve or mix the sugar solution.

D-4.5 Filteration and Incubation

Connect the filter apparatus to the vacuum pump. If not already sterile, flame the filter funnel and base with alcohol. After cooling place the membrane centrally on the base with sterile forceps. Place the sterile funnel on the top and clamp in place.

Pour the sample into the funnel, apply the vacuum and filter the sample. Rinse the funnel with sterile water. Turn off the vacuum. Remove the funnel and use flamed forceps to place the membrane on an agar plate or moistened nutrient pad. When positioning the membrane on the culture plate ensure that no air bubbles are trapped between them.

Invert the dishes and incubate at 30°C for 48 h.

D-5 CALCULATION

Count the colonies on each membrane using the colony counting equipment. For membranes showing more than 100 colonies the test should be repeated using a lower dilution of the sample.

Calculate the number of CFU dry matter by the following calculation:

$$\frac{\Sigma c}{n} = \text{CFU/10 g}$$

where

Σc = sum of the colonies on all plates; and

n = number of plates.

ANNEX E

(*Clause* 8)

SAMPLING OF LIQUID SUGAR

E-1 GENERAL REQUIREMENTS FOR SAMPLING

E-1.0 In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed.

E-1.1 Sampling instrument shall be clean and dry when used.

E-1.2 Precautions shall be taken to draw the samples in a manner so as to protect the samples, the material being sampled, the sampling instrument and the containers for samples from adventitious contamination.

E-1.3 The samples shall be placed in clean and dry glass containers.

E-1.4 The sample containers shall be sealed air-tight after filling and marked with full details of sampling, date of manufacture, name of manufacturer and other important particulars of the consignment.

E-1.5 Samples shall be stored in such a manner that the conditions of storage do not unduly affect the quality of the material.

E-2 SCALE OF SAMPLING

E-2.1 Lot

All the containers in a single consignment of the material of the same grade, drawn from a single batch of manufacture shall constitute a lot. If a consignment is declared to consist of different batches of manufacture, the containers belonging to the same grade and batch shall be grouped together. Each group shall constitute separate lots.

E-2.2 Gross Sample

For the purpose of drawing samples for test, one container from each lot shall be selected by the purchaser. This container shall constitute the gross sample.

E-2.2.1 The container selected for the gross sample shall not be damaged or leaky and shall have its seal intact.

E-3 PROCEDURE FOR DRAWING A SAMPLE FROM THE CONTAINER

E-3.1 Sampling Pump

For the purpose of drawing a sample of liquid sugar from the container, it is recommended that a pump of the type shown in Fig. 1 may be used. The pump is made of copper. The tube of the pump is 2 mm thick, with an internal diameter of 42 mm. The tube is provided with a downward bent spout at 76 mm from the upper end of the screw top. The spout is of 19 mm internal diameter. The piston is provided with lifting up valves. The piston rod is 8 mm in diameter and is made of copper. The rod is provided with a locking device at its top so that it may not have to be kept pressed down while the material rises up inside the tube.

E-3.2 Method of Sampling

Place the container selected for the purpose of sampling at a clean and dry place. Thoroughly clean the lid of the opening of the container and the area round about the opening with soap and water. Wipe the cleaned area dry. Remove the lid of the opening and take out with a ladle sufficient quantity of the material to remove the top scum, if any. Insert the pump (*see* **E-3.1**) with its piston locked in position, through the opening of the container and very slowly press the pump down to the extent of the upper one-third height of the container. Hold the pump in this position for 5 min. Again very slowly press the pump down through the middle one-third height of the container and hold the pump in that position for 5 min. Press the pump down again very slowly and when it touches the bottom of the container, raise it a little so that it is just above the bottom of the container. Allow the pump to remain in that position again for 5 min (The pump shall have been filled in by this time). Take out the pump and wipe its outside surface so as to remove the material which may be adhering to it. Unlock the piston and work it very slowly through one complete upward movement, simultaneously, collecting the material flowing out of the spout in a perfectly clean and dry sample container. Repeat the operation of drawing samples in the above manner, until a quantity of about 2 kg of the material is collected in the sample container.

E-4 TEST SAMPLES AND REFEREE SAMPLE

E-4.1 Preparation of Test Samples

To prepare test samples, thoroughly mix the material drawn as specified under **E-3.2** and divide it into three equal parts, each part (test sample) being not less than 0.6 kg. Transfer these test samples to thoroughly clean and dry sample containers, and seal them air-tight. Label the sample containers with all the particulars given under **E-1.4**. One of these samples shall be sent to the purchaser and one to the vendor.

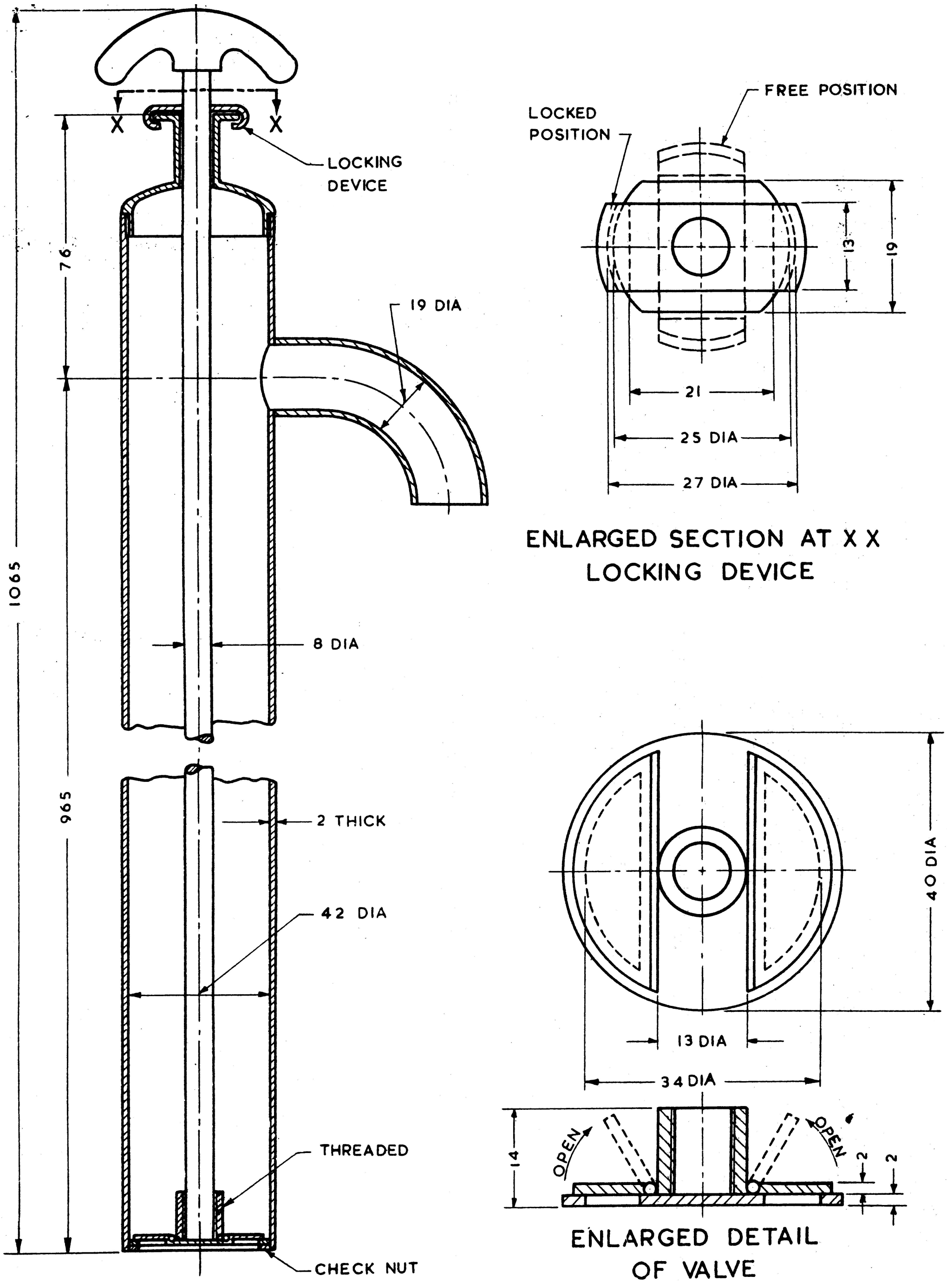
E-4.2 Referee Sample

The third sample, bearing the seals of the purchaser and the vendor, shall constitute the referee sample to be used in case of dispute between the purchaser and the vendor. It shall be kept at a place agreed to between the purchaser and the vendor.

E-5 NUMBER OF TESTS AND CRITERIA FOR CONFORMITY

E-5.1 The test sample as prepared under **E-3.2** shall be tested for all characteristics (*see* **5**).

E-5.2 The lot shall be declared as conforming to this specification, if the test results satisfy the requirements of each of the characteristics.



All dimensions in millimetres.

FIG. 1 SAMPLING PUMP

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